ASSOCIATION BETWEEN ATP CITRATE LYASE (*ACLY*) GENE POLYMORPHISM AND FATTENING, SLAUGHTER AND PORK QUALITY TRAITS IN POLISH PIGS*

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Abstract

The primary aim of this study was to estimate the relationship between ATP citrate lyase (*ACLY*) gene polymorphism (c.*523 T>C) and fattening and pork quality traits. Investigations were carried out on 526 pigs represented by three breeds: Polish Landrace (n=269), Polish Large White (n=189) and Puławska (n=68). *ACLY* genotypes were determined by PCR–RFLP method. It was demonstrated that the analyzed polymorphism had significant influence ($P<0.05$ and $P\leq 0.01$) on several economically important traits in pigs, e.g. average daily gain, average backfat thickness, lean meat percentage. The results obtained allow for application of c.*523 T>C polymorphism in breeding programs to improve the pig population in terms of fattening and slaughter traits. However, this breeding program may have a slight negative effect on meat texture parameters.

Key words: pigs, ATP citrate lyase gene, gene polymorphism, fattening and slaughter traits, meat quality

Pigs are an important source of meat in many countries and provide ~36% of the total meat consumption in the world (URL1, URL2). For many years, breeding programs have focused on improving the pig's fattening and slaughter traits and their interrelationships (Żak et al., 2008, 2009). Currently, increased attention is focused on improving the quality of meat, because of growing awareness of consumers re-

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garding its safety and influence on human health. The overall selection goal in modern breeding programs is directed to improving qualitative and quantitative features that are economically important in pork production. As pork quality is influenced by many genetic and non-genetic factors, many studies have been focused on revealing the genetic background and its various genetic factors (Guy et al., 2012; Piórkowska et al., 2018; Ropka-Molik et al., 2018; Catillo et al., 2020). Increases in societal pressure for sustainable pork production highlight the need for alternative, more holistic approaches in genetic selection programs, that will enable the production of high-quality meat, while maintaining performance and carcass characteristics of pigs at a satisfactory level (Merks et al., 2012).

Meat and carcass quality traits are influenced by many parameters, but especially by fat content. The fatty acid composition (FAC) in the pig carcass and meat is an important factor in determining pork quality (Davoli et al., 2019). Lipogenesis is a complex metabolic process that involves many enzymes. For example, ATP citrate lyase (ACLY) is important in fatty acid biosynthesis (Davoli et al., 2014). The cytosolic enzyme, ACLY, converts mitochondria-derived citrate into acetyl CoA. It catalyzes the formation of acetyl-CoA and oxaloacetate from citrate and CoA with simultaneous hydrolysis of ATP to ADP and phosphate. Acetyl CoA is a vital building block for the endogenous biosynthesis of fatty acids (Zaidi et al., 2012) and is involved in lipogenesis and cholesterologenesis (Chypre et al., 2012).

The *ACLY* gene is localized on the porcine chromosome 12 (SSC12) (NCBI, Gene ID: 100125957). Several quantitative trait loci (QTLs) affecting pork quality have been reported in SSC12 (Uemoto et al., 2009). The chromosome (SSC12) is relevant to the genetics of fatty acids (FA) composition because of genes involved in the biosynthesis of FA (Clöp et al., 2003; Muñoz et al., 2007). Some functional candidate genes (*ACLY*, *ACOX1*, *SREBF1*, *ACACA*) have been analyzed for explaining the QTL effects (Muñoz et al., 2013; Piórkowska et al., 2020). Davoli et al. (2014) reported that the *ACLY* gene could be taken into consideration as a marker in genetic programs aimed to modify the fat content in muscle. Recently, Shang et al. (2019) revealed that the *ACLY* gene was responsible for fatness and FA composition traits in Tibetan and Yorkshire pigs. The *ACLY* gene was also associated with growth traits and fatty acid content of milk in cattle (Bouwman et al., 2011; Li et al., 2016).

Muñoz et al. (2013) detected two single nucleotide polymorphisms (SNPs) in porcine *ACLY* gene – c.2970T > C and c.*523 T>C. First one is a silent variant located in exon 24, however the second one is located in the 3'-UTR region. Genotyping of animals from an experimental F₂ cross between Iberian and Landrace pig lines revealed that SNP c.*523 T>C was the most informative in this population showing intermediate allelic frequencies. UTRs jointly with other factors interacting with RNA may be responsible for stability, transport, and sub-cellular localization of mRNA as well as the efficiency of translation, which affects the total amount of synthesized protein (Matoulkova et al., 2012). Polymorphism located in this area can influence all the mechanisms mentioned above and in turn may affect phenotypic traits in animals, especially with high economic importance. Therefore the aim of this study was to estimate associations between *ACLY* polymorphism c.*523 T>C and fattening, slaughter and meat quality traits in three Polish pig breeds.

Material and methods

Ethics statement

The bioethic committee approval of this study is not necessary because the experiment was carried out on biological material derived from the Pig Testing Stations of the National Research Institute of Animal Production where pigs were fed, maintained and slaughtered in standardized conditions to estimate their performance and breeding values.

Animals and feeding

The study utilized 526 sows from three breeds: Polish Landrace (n=269), Polish Large White (n=189) and native breed Puławska (n=68). The animals were from 116 fathers and 248 mothers. On average, 4.53 animals came from 1 father and 1.51 animals from 1 mother. Animals were maintained in Pig Testing Stations (National Research Institute of Animal Production) located in Poland. Feeding and housing conditions were consistent for all animals. Pigs were housed from 30 kg body weight and kept in individual pens. They were fed *ad libitum* with two mixtures until they reached 100 (± 2.5) kg, at which point they were slaughtered by exsanguination after stunning with high-voltage electric tongs. For further analysis samples from *longissimus thoracis et lumborum* (LTL) and *semimembranosus* (SM) muscles were collected. Genomic DNA was extracted from *longissimus thoracis et lumborum*. The LTL and SM muscles were used to measure the texture parameters.

Genotyping

Genomic DNA was extracted from muscle tissue using ReliaPrep™ gDNA Tissue Miniprep System (Promega, USA), Genomic Mini (A&A Biotechnology, Poland) and Sherlock AX (A&A Biotechnology, Poland). DNA was isolated in several steps. We used kits which were actually available at the Department. Genotypes of *ACLY* gene were analyzed using the PCR-RFLP method. Genetic variants of the *ACLY* gene (c.*523 T>C) were identified with the following primer sequences (Davoli et al., 2014):

forward 5'–TGG AAG CAT CTA AGC ACT GG–3'

reverse 5'–CCT GAC ACA AAG AGA CAC TGG–3'

Each PCR reaction was performed in a total volume of 15 μ l containing 7.5 μ l of 2xPCR Mix™ (A&A Biotechnology, Poland), 1 μ l of each primer (10 pmol), 4.5 μ l of water and 1 μ l of DNA (~20 ng/ μ l). Thermal conditions were as follows: 95°C for 5 min, followed by 34 cycles of 95°C for 30 s, 64°C for 30 s and 72°C for 30 s and a final extension step at 72°C for 5 min, and completed by cooling for 10 min, at 4°C. Obtained amplicons (319 base pair, bp) were digested with *BsrDI* (Thermo Fisher Scientific, USA) for 3 hours at 65°C. The restriction fragments were separated in a 2.5% agarose gel stained with ethidium bromide in 1×TBE (120 V, 40 min) and visualized in UV light (Vilber Lourmat, France).

Performance test

During the performance experiment at Pig Testing Stations, the following fattening traits were recorded: average daily gain in station test from 30 to 100 kg body

weight (g), lifetime daily gain (g), feed conversion ratio (kg/kg gain), daily feed intake (kg), age at slaughter (days), and number of days on test (days).

Carcass and meat quality traits

After slaughter, dissection was performed and the following traits were measured: carcass yield (%), weights of loin and ham without backfat and skin (kg), average backfat thickness (cm), loin eye width (cm), length (cm) and area (cm²), lean meat percentage (%) and weight of primary cuts (kg). Average backfat thickness was measured at 5 points: the thickest point over the shoulder; on the back above the joint between the last thoracic and first lumbar vertebrae; at three points over the loin: above the rostral edge, above the middle and above the caudal edge of gluteal muscle cross-section according to Tyra and Žak (2012). To determine the loin area, the muscle was cut between the last thoracic and the first lumbar vertebra and then was measured based on the contour made on the cephalic plane.

For meat quality, the following traits were estimated: intramuscular fat content (IMF), meat colour, pH and water exudation (%) and texture parameters. For IMF content assessment, samples were collected from the middle cross sectional area of *m. longissimus dorsi*, cut behind the last rib. Thawed muscles were homogenized and crude fat was extracted by Soxhlet method using Soxtherm SOX 406 (Gerhardt, Germany) system (Oczkowicz et al., 2012). The pH was determined 45 min (pH45) and 24 hours (pH24) after slaughter on hanging carcasses in *longissimus lumborum* and *semimembranosus* muscles by means of pH-Star pH meter (Matthäus, Germany). Three colour parameters of loin – luminosity (L*), redness (a*), yellowness (b*) – were measured by use of CR-310 chroma meter (Minolta Konica, Japan). Water exudation was determined as the amount of free water according to the Grau-Hamm method (filter paper press) (Grau and Hamm, 1953).

To measure the texture of *longissimus lumborum* and *semimembranosus* muscles two methods were used – shear force test and texture profile analysis test (TPA). In case of the cutting test, two traits were determined – firmness and toughness. Shear force was determined on raw and cooked muscles using TA.XTPlus texture analyser (Stable Micro Systems, UK) with Warner-Bratzler triangular blade. For texture profile analysis, the following traits were determined: hardness, springiness, cohesiveness, chewiness, elasticity. The TPA was performed using the same instrument equipped with a 50 mm cylinder. All the parameters were calculated according to the procedure given by Ropka-Molik et al. (2014).

Statistical analyses

All analyzed traits were assessed in a fixed model using the least squares method of the GLM (General Linear Model) procedure in the SAS software (Ver. 8.02). The model was as follows:

$$Y_{ijk} = \mu + b_i + g_j + (bg)_{ij} + \beta SW_k + e_{ijk}$$

where:

Y_{ijk} – estimated trait,

μ – the overall mean of the trait,
 b_i – the fixed effect of i breed;
 g_j – the fixed effect of j genotype of *ACLY* gene,
 $(bg)_{ij}$ – interaction between i breed and j genotype of *ACLY* gene,
 βSW_k – linear effect of slaughter weight as covariate (for slaughter traits only),
 e_{ijk} – random residual error.

The Least Square Means (LSM) method was used for determination of significant differences between genotype groups. The differences in phenotype in particular genotype groups were presented in tables as $LSM \pm SE$.

Results

Digestion of PCR amplicons (319 bp) with *Bsr*DI enzyme allowed determination of three genotypes, based on the following restriction fragments lengths: *TT* – 319 bp, *CC* – 168 and 151 bp and *TC* – 319, 168 and 151 bp. The present study shows similar frequency of *ACLY* (c.*523 T>C) genotypes and alleles between the breeds investigated (Table 1). In all groups, the heterozygous genotype was the most frequent, while the homozygous *CC* genotype occurred with lowest frequency. The high number of heterozygotes in our research may be due to the fact that the samples were randomly selected. Moreover, the material was derived from relatively short period of time in order to minimize additional effects which could affect the analyzed traits. In Puławska breed the *CC* genotype was not observed. It represents Polish native breed under protection program and frequency of genotypes and alleles is often different than in high-productive breeds. The frequencies of allele *T* in this study (0.54–0.55) were slightly different than reported for Italian Large White (0.468) and Italian Duroc (0.754) pigs (Davoli et al., 2014).

Table 1. Genotype and allele frequencies with Hardy–Weinberg statistics calculated for the *ACLY* SNP (c.*523 T>C)

Breed	n	Genotype			Allele		HWE	
		<i>TT</i>	<i>TC</i>	<i>CC</i>	<i>T</i>	<i>C</i>	χ^2	<i>P</i>
Polish Landrace	269	0.14 (n=38)	0.82 (n=220)	0.04 (n=11)	0.55	0.45	114.47	$P \leq 0.01$
Polish Large White	189	0.12 (n=22)	0.84 (n=159)	0.04 (n=8)	0.54	0.46	90.46	$P \leq 0.01$
Puławska	68	0.07 (n=5)	0.93 (n=63)	-	0.54	0.46	50.65	$P \leq 0.01$

n — the number of animals, HWE – Hardy–Weinberg equilibrium.

Distribution of the *ACLY* genotypes was not in the state of Hardy-Weinberg equilibrium in all analyzed breeds. This is probably caused by long lasting selection of these breeds, which disturbed normal distribution of *ACLY* genotypes.

The present research showed the significant associations between c.*523 T>C polymorphism in *ACLY* gene with fattening, slaughter and pork quality traits in Polish pigs (Tables 2 to 6).

Table 2. Association between *ACLY* polymorphism (c.*523 T>C) and fattening performance traits in analyzed breeds (LSM $\pm$ SE)

Trait	Genotype	Polish Landrace (n=269)	Polish Large White (n=189)	Puławska (n=68)
Average daily gain in station test (g)	CC	928 $\pm$ 36.5 a	897 $\pm$ 35.6	–
	TC	918 $\pm$ 24.6	889 $\pm$ 16.0	808 $\pm$ 10.5 A
	TT	909 $\pm$ 27.8 b	903 $\pm$ 25.9	738 $\pm$ 33.6 B
Lifetime daily gain (g)	CC	617 $\pm$ 26.6	610 $\pm$ 17.1 a	–
	TC	617 $\pm$ 17.9	587 $\pm$ 7.7	559 $\pm$ 8.2
	TT	617 $\pm$ 20.2	584 $\pm$ 12.4 b	542 $\pm$ 26.3
Feed conversion ratio (kg/kg gain)	CC	2.598 $\pm$ 0.096	2.621 $\pm$ 0.070	–
	TC	2.638 $\pm$ 0.065	2.687 $\pm$ 0.032	2.770 $\pm$ 0.027 a
	TT	2.675 $\pm$ 0.073	2.739 $\pm$ 0.051	2.934 $\pm$ 0.088 b
Daily feed intake (kg)	CC	2.398 $\pm$ 0.098	2.343 $\pm$ 0.086	–
	TC	2.406 $\pm$ 0.066	2.377 $\pm$ 0.039	2.259 $\pm$ 0.021
	TT	2.424 $\pm$ 0.075	2.465 $\pm$ 0.062	2.156 $\pm$ 0.066
Age at slaughter (days)	CC	172 $\pm$ 7.94	169 $\pm$ 5.12	–
	TC	171 $\pm$ 5.37	176 $\pm$ 2.30	181 $\pm$ 2.75
	TT	172 $\pm$ 6.04	174 $\pm$ 3.72	184 $\pm$ 8.80
Number of days on test (days)	CC	80.5 $\pm$ 3.40	81.9 $\pm$ 3.08	–
	TC	82.5 $\pm$ 2.30	82.8 $\pm$ 1.39	86.5 $\pm$ 1.25 a
	TT	82.9 $\pm$ 2.59	81.6 $\pm$ 2.24	94.9 $\pm$ 4.01 b

LSM – least squares mean; standard error (SE); n – the number of animals; values marked with different letter indicate statistically significant differences: $P < 0.05$ (abc) or $P \leq 0.01$ (ABC).

The GLM procedure indicated the significant association of c.*523 T>C with improved daily gain in Polish Landrace (CC>TT; $p < 0.05$) and Puławska (TC>TT; $P \leq 0.01$), lifetime daily gain (CC>TT; $P < 0.05$) in Polish Large White and feed conversion and number of days on test (TT>TC; $P < 0.05$) in Puławska (Table 2). The *ACLY* polymorphism was associated ($P < 0.05$) with carcass yield (in Puławska; TC>TT), weight of loin without backfat and skin (in Polish Landrace; TT>CC), weight of ham without backfat and skin (in Puławska, TT>TC), average backfat thickness from 5 measurements (Polish Landrace and Polish Large White; TT, TC>CC and TT>CC respectively), loin eye area (Polish Large White, CC>TT), lean meat percentage (Polish Landrace and Polish Large White; CC>TT and CC>TC, TT respectively) and weight of primary cuts (Polish Large White; CC>TT) – Table 3. For meat colour traits (Table 4), statistically significant differences ($P < 0.05$) between CC and TT animals were observed in Polish Large White for luminosity (CC>TT) and yellowness trait (TT>CC). In the Polish Landrace breed statistical difference was found between CC and TC genotypes only for yellowness of meat (TC>CC).

Table 3. Association between *ACLY* polymorphism (c.*523 T>C) and carcass traits in analyzed breeds (LSM $\pm$ SE)

Trait	Genotype	Polish Landrace (n=269)	Polish Large White (n=189)	Puławska (n=68)
Carcass yield (%)	CC	77.0 $\pm$ 0.87	76.5 $\pm$ 0.88	—
	TC	78.1 $\pm$ 0.59	77.1 $\pm$ 0.40	76.0 $\pm$ 0.39 a
	TT	77.8 $\pm$ 0.66	76.2 $\pm$ 0.64	74.5 $\pm$ 1.26 b
Weight of loin without backfat and skin (kg)	CC	5.97 $\pm$ 0.28 a	6.19 $\pm$ 0.21	—
	TC	6.55 $\pm$ 0.19 b	6.33 $\pm$ 0.10	5.861 $\pm$ 0.09
	TT	6.38 $\pm$ 0.21 b	6.17 $\pm$ 0.15	6.072 $\pm$ 0.28
Weight of ham without backfat and skin (kg)	CC	9.37 $\pm$ 0.22	9.24 $\pm$ 0.19	—
	TC	9.19 $\pm$ 0.15	9.08 $\pm$ 0.08	9.05 $\pm$ 0.10 a
	TT	9.28 $\pm$ 0.17	9.03 $\pm$ 0.14	8.76 $\pm$ 0.31 b
Average backfat thickness from 5 measurements (cm)	CC	1.34 $\pm$ 0.12 a	1.29 $\pm$ 0.09 a	—
	TC	1.55 $\pm$ 0.08 b	1.33 $\pm$ 0.04	1.41 $\pm$ 0.04
	TT	1.48 $\pm$ 0.09 b	1.39 $\pm$ 0.06 b	1.36 $\pm$ 0.14
Loin eye width (cm)	CC	10.50 $\pm$ 0.292	10.28 $\pm$ 0.337	—
	TC	10.44 $\pm$ 0.197	10.12 $\pm$ 0.153	10.24 $\pm$ 0.111
	TT	10.52 $\pm$ 0.222	10.35 $\pm$ 0.242	10.18 $\pm$ 0.369
Loin eye length (cm)	CC	6.66 $\pm$ 0.264	6.83 $\pm$ 0.208	—
	TC	6.73 $\pm$ 0.178	6.89 $\pm$ 0.094	6.84 $\pm$ 0.080
	TT	7.02 $\pm$ 0.201	6.87 $\pm$ 0.245	7.06 $\pm$ 0.259
Loin eye area (cm ²)	CC	54.2 $\pm$ 2.05	54.4 $\pm$ 1.87 a	—
	TC	55.6 $\pm$ 1.38	53.6 $\pm$ 0.84 b	52.4 $\pm$ 0.86
	TT	55.1 $\pm$ 1.56	53.4 $\pm$ 1.36 b	53.8 $\pm$ 2.75
Lean meat percentage (%)	CC	61.0 $\pm$ 1.17 a	61.1 $\pm$ 1.03 a	—
	TC	59.9 $\pm$ 0.79	60.3 $\pm$ 0.46 b	61.3 $\pm$ 0.38
	TT	60.2 $\pm$ 0.89 b	60.0 $\pm$ 0.75 b	61.9 $\pm$ 1.21
Weight of primary cuts (kg)	CC	24.3 $\pm$ 0.50	24.1 $\pm$ 0.44 a	—
	TC	24.5 $\pm$ 0.34	23.9 $\pm$ 0.20	23.1 $\pm$ 0.22
	TT	24.5 $\pm$ 0.38	23.7 $\pm$ 0.32 b	23.1 $\pm$ 0.72

LSM – least squares mean; standard error (SE); n – the number of animals; values marked with different letter indicate statistically significant differences: $P < 0.05$ (abc).

In Polish Landrace pigs, the *m. longissimus lumborum* from pigs with CC genotype had higher values of toughness (raw) ($P < 0.05$), firmness ($P \leq 0.01$) and toughness ($P \leq 0.01$) than TC and TT (Table 5). The same trend of associations was observed in Polish Large White (higher values of firmness ($P < 0.05$) and toughness ($P \leq 0.01$) for *m. longissimus lumborum* in CC individuals than TC and TT ones). The influence of *ACLY* genotype for *m. longissimus lumborum* texture parameters of Puławska breed was observed only for toughness (higher values for TC than TT, $P \leq 0.01$). The analysis of associations between *semimembranosus* texture traits and *ACLY* polymorphism (Table 6) showed higher values of toughness and chewiness of meat from CC pigs than from TC and TT pigs (Large White ($P \leq 0.01$) and Landrace ($P < 0.05$; $P \leq 0.01$)) and higher values ($P < 0.05$) of firmness (raw), firmness and chewiness in the meat from TC animals compared to TT in Puławska breed.

Table 4. Association between *ACLY* polymorphism (c.*523 T>C) and meat quality traits in analyzed breeds (LSM $\pm$ SE)

Trait	Genotype	Polish Landrace (n=269)	Polish Large White (n=189)	Puławska (n=68)
IMF	CC	1.140 $\pm$ 0.06	1.178 $\pm$ 0.12	–
	TC	1.161 $\pm$ 0.04	1.200 $\pm$ 0.05	1.206 $\pm$ 0.02
	TT	1.143 $\pm$ 0.05	1.215 $\pm$ 0.08	1.247 $\pm$ 0.06
Meat colour (L*)	CC	53.1 $\pm$ 1.12	54.8 $\pm$ 0.68 a	–
	TC	54.7 $\pm$ 0.76	53.8 $\pm$ 0.31	53.9 $\pm$ 0.26
	TT	52.4 $\pm$ 0.86	52.1 $\pm$ 0.50 b	54.0 $\pm$ 0.83
Meat colour (a*)	CC	16.7 $\pm$ 0.77	17.43 $\pm$ 0.47	–
	TC	17.4 $\pm$ 0.52	17.92 $\pm$ 0.21	16.8 $\pm$ 0.14
	TT	17.8 $\pm$ 0.58	18.26 $\pm$ 0.34	16.1 $\pm$ 0.46
Meat colour (b*)	CC	2.665 $\pm$ 0.63 a	2.323 $\pm$ 0.40 a	–
	TC	3.551 $\pm$ 0.42 b	3.600 $\pm$ 0.18	2.289 $\pm$ 0.08
	TT	3.501 $\pm$ 0.48	3.643 $\pm$ 0.29 b	2.178 $\pm$ 0.27
pH45 (LD)	CC	6.294 $\pm$ 0.08	6.240 $\pm$ 0.07	–
	TC	6.329 $\pm$ 0.05	6.273 $\pm$ 0.03	6.287 $\pm$ 0.02
	TT	6.313 $\pm$ 0.062	6.237 $\pm$ 0.05	6.267 $\pm$ 0.07
pH24 (LD)	CC	5.473 $\pm$ 0.04	5.527 $\pm$ 0.03	–
	TC	5.546 $\pm$ 0.03	5.537 $\pm$ 0.01	5.635 $\pm$ 0.01
	TT	5.512 $\pm$ 0.03	5.554 $\pm$ 0.02	5.573 $\pm$ 0.03
pH45 (SM)	CC	6.420 $\pm$ 0.07	6.205 $\pm$ 0.07	–
	TC	6.474 $\pm$ 0.05	6.396 $\pm$ 0.03	6.266 $\pm$ 0.02
	TT	6.485 $\pm$ 0.05	6.368 $\pm$ 0.05	6.319 $\pm$ 0.06
pH24 (SM)	CC	5.544 $\pm$ 0.04	5.573 $\pm$ 0.04	–
	TC	5.600 $\pm$ 0.03	5.599 $\pm$ 0.02	5.650 $\pm$ 0.02
	TT	5.586 $\pm$ 0.03	5.641 $\pm$ 0.03	5.603 $\pm$ 0.06
Water exudation (%)	CC	32.4 $\pm$ 2.48	33.8 $\pm$ 2.12	–
	TC	33.2 $\pm$ 1.70	32.8 $\pm$ 1.03	34.0 $\pm$ 0.88
	TT	35.7 $\pm$ 1.91	30.8 $\pm$ 1.60	31.3 $\pm$ 2.81

LSM – least squares mean; standard error (SE); IMF – intramuscular fat content; L* – luminosity; a* – redness; b* – yellowness; pH45, pH24 – pH measured 45 min and 24 h after slaughter, respectively; LD – *m. longissimus dorsi*; SM – *m. semimembranosus*; n – the number of animals; values marked with different letter indicate statistically significant differences: P<0.05 (abc).

Table 5. Association between *ACLY* polymorphism (c.*523 T>C) and *longissimus lumborum* texture parameters in analyzed breeds (LSM $\pm$ SE)

Trait	Genotype	Polish Landrace (n=269)	Polish Large White (n=189)	Puławska (n=68)
1	2	3	4	5
Firmness (r)	CC	31.6 $\pm$ 4.84	35.4 $\pm$ 3.79	–
	TC	29.1 $\pm$ 3.12	32.1 $\pm$ 1.62	18.26 $\pm$ 1.28
	TT	29.6 $\pm$ 3.57	30.9 $\pm$ 2.72	18.50 $\pm$ 3.84
Toughness (r)	CC	89.5 $\pm$ 13.46 a	91.6 $\pm$ 11.10	–
	TC	83.9 $\pm$ 8.67 b	88.2 $\pm$ 4.73	50.8 $\pm$ 3.87
	TT	85.1 $\pm$ 9.94 b	83.8 $\pm$ 7.96	50.2 $\pm$ 11.60

Table 5 – contd.

1	2	3	4	5
Firmness	CC	109.0±8.94 A	98.9±8.31 a	–
	TC	93.9±5.76 B	92.0±3.55 b	75.4±2.53
	TT	93.6±6.60 B	92.9±5.96 b	69.4±7.60
Toughness	CC	244±22.6 A	248±23.2 A	–
	TC	220±14.6 B	221±9.9 B	178±6.7 A
	TT	225±16.7 B	221±16.6 B	151±20.1 B
Hardness	CC	9.87±1.54	12.23±1.51	–
	TC	9.43±0.99	11.22±0.64	6.81±0.51
	TT	9.40±1.14	12.24±1.08	6.74±1.53
Springiness	CC	0.694±0.027	0.725±0.025	–
	TC	0.692±0.017	0.731±0.011	0.684±0.009
	TT	0.699±0.020	0.725±0.018	0.685±0.027
Cohesiveness	CC	0.643±0.025	0.674±0.022	–
	TC	0.651±0.016	0.679±0.009	0.626±0.008
	TT	0.660±0.019	0.664±0.016	0.640±0.025
Chewiness	CC	4.295±0.76	6.186±0.72	–
	TC	4.355±0.49	5.672±0.31	3.077±0.26
	TT	4.469±0.56	6.052±0.51	2.926±0.77
Elasticity	CC	0.273±0.015	0.304±0.013	–
	TC	0.284±0.010	0.302±0.006	0.266±0.005
	TT	0.291±0.011	0.295±0.009	0.275±0.016

LSM – least squares mean; standard error (SE); r – raw tissue, n – the number of animals; values marked with different letter indicate statistically significant differences: $P < 0.05$ (abc) or $P \leq 0.01$ (ABC).

Table 6. Association between *ACLY* polymorphism (c.*523 T>C) and *semimembranosus* texture parameters in analyzed breeds (LSM ± SE)

Trait	Genotype	Polish Landrace (n=269)	Polish Large White (n=189)	Puławska (n=68)
1	2	3	4	5
Firmness (r)	CC	24.2±2.88	28.9±2.64	–
	TC	24.8±1.85	26.9±1.44	23.7±0.73 a
	TT	25.0±2.13	27.0±2.03	20.0±2.34 b
Toughness (r)	CC	79.0±9.77	78.3±9.21	–
	TC	77.9±6.27	79.7±5.01	68.0±2.32
	TT	77.3±7.21	80.7±7.01	54.0±7.41
Firmness	CC	85.9±9.48	93.0±9.77	–
	TC	88.6±6.09	93.3±5.32	94.0±3.09 a
	TT	87.4±7.00	92.8±7.49	90.0±9.87 b
Toughness	CC	258±21.8 a	238±25.8 A	–
	TC	231±14.0 b	227±14.0 B	199±6.5
	TT	231±16.1 b	231±19.8 B	201±20.6
Hardness	CC	18.20±3.60	11.42±1.73	–
	TC	25.20±1.68	11.13±0.94	10.35±0.53
	TT	17.00±2.60	12.36±1.33	9.77±1.71
Springiness	CC	0.787±0.038	0.766±0.038	–
	TC	0.774±0.024	0.769±0.021	0.724±0.011
	TT	0.764±0.028	0.784±0.029	0.695±0.034

Table 6 – contd.

1	2	3	4	5
Cohesiveness	CC	0.687±0.032	0.762±0.191	–
	TC	0.682±0.020	0.762±0.191	0.625±0.011
	TT	0.680±0.024	0.702±0.146	0.597±0.035
Chewiness	CC	5.86±1.03 Aa	6.46±0.89 A	–
	TC	4.96±0.66 Ab	5.75±0.48 B	4.795±0.303 a
	TT	4.33±0.76 B	5.36±0.68 B	4.153±0.967 b
Elasticity	CC	0.302±0.017	0.292±0.014	–
	TC	0.297±0.011	0.297±0.008	0.256±0.006
	TT	0.304±0.013	0.290±0.011	0.248±0.019

LSM – least squares mean; standard error (SE); r – raw tissue, n – the number of animals; values marked with different letter indicate statistically significant differences: $P < 0.05$ (abc) or $P \leq 0.01$ (ABC).

Discussion

The studies showed a positive effect of the CC genotype of the *ACLY* gene on the fattening and slaughter traits of pigs.

Similar results were reported by Davoli et al. (2014). The authors investigated two SNPs in the *ACLY* gene in relation to growth and carcass traits in Italian Duroc and Italian Large White pigs. They found that Italian Large White individuals carrying the CC genotype had the highest growth rates, the highest lean cut weights, the heaviest hams and the lowest backfat thickness.

We found that in Puławska breed TC genotype is associated with better values of fattening performance, carcass traits and meat quality. However, in the case of Polish Landrace and Polish Large White the same tendency was observed for CC genotype. These differences may result from the different utility types of analyzed pigs. Puławska is a local breed, covered by the Genetic Resources Protection Program and represents the type between fatty-meat and meat. Other two breeds, instead are highly productive and classified as a meat type (Szyndler-Nędza et al., 2019).

The obtained results concerning effects of *ACLY* c.*523 T>C polymorphism on fattening and meat quality traits fit in with the research trend of other authors who investigated this gene. The c.*523 T>C polymorphism is located in 3'UTR region of *ACLY* gene and may differentiate the regulation of its expression via non-coding RNAs. The *ACLY* gene is involved in many biological processes. Zhang et al. (2020) analyzed the expression profiles of non-coding RNAs in osteoporosis and found that *ACLY* is a key hub gene in the protein-protein network (PPI). Gan et al. (2018) found the influence of miR-224-5p expression on prostate cancer development via *ACLY* target gene, one of the key elements in the PPI network. Similarly, Cheng et al. (2017) indicated that miR-133b targets *ACLY* and inhibits gastric cancer cell proliferation. Rayner et al. (2011) found that miR-33 reduced genes involved in fatty acid synthesis (*SREBF1*, *FASN*, *ACLY*, *ACACA*), resulting in a marked suppression of plasma triglyceride levels. In another study (Shang et al., 2019), different expression profiles of lncRNAs in association with fatness and fatty acid composition traits in

pigs were reported. The authors revealed that *ACLY* and other genes (responsible for higher monounsaturated fatty acid, octadecenoic acid, exadecenoic acid, and hexadecanoic acids) are the target genes for some lncRNAs.

Bouchard-Mercier et al. (2013) showed an association between rs8071753 within *ACLY* gene and plasma triglyceride concentrations response following n-3 PUFA supplementation. An analysis showed that within the 3'-UTR of porcine *ACLY* gene potential target sites for ssc-miR-376c and ssc-miR-376b are present but their locations are not covered with analyzed SNP (miRBase, Kozomara et al., 2019). In the neighborhood of c.*523 T>C variant polyadenylation signal and Musashi binding element are located (RegRNA 2.0, Chang et al., 2013). MacNicol et al. (2011) showed that Musashi acts as an activator of translation or repressor of target mRNA translation depending on the type of cells and species. Interestingly Musashi can be converted from a repressor of mRNA translation to an activator of translation in response to extracellular stimuli. However, Davoli et al. (2014) found differences in *ACLY* mRNA relative abundance between Duroc and Large White breeds, but no differences in gene expression levels among the different c.*523 T>C genotypes in muscle and back fat tissue. The authors suggested that c.*523 T>C may be linked with causative mutation of another unidentified gene, influencing the growth and carcass quality traits.

Conclusions

Summing up, at the present stage of study, the obtained results could suggest the possibility of using the c.*523 T>C polymorphism in the *ACLY* gene as a genetic marker in some pig breeds reared in Poland for several traits that are important from an economic point of view (C>T improved growth rate, average daily gains, average backfat thickness, lean meat percentage), especially for high-production breeds. However, the low frequency of the beneficial allele in individual breeds, low effect on the traits mentioned above and the lack of unambiguous direction of interaction in individual breeds indicate that its practical use may be limited. The effects we observed are probably due to indirect influence of *ACLY* gene on the analyzed production traits. It may suggest the further search for other gene in the neighborhood of this *locus* with causative effect on these traits.

Author contributions

Conceptualization, A.T.; methodology, K.W-M.; A.K.; D.P.; M.T.; M.S-N.; G.Ż.; validation, M.S.-N.; formal analysis, A.R.; investigation, M.S.-N.; data curation, M.T.; writing – original draft preparation, K.W-M.; A.K.; A.T.; A.D.; D.P.; writing – review and editing, A.D.; D.P.; M.T.; M.S-N.; G.Ż.; supervision – A.D.

Conflicts of interest

The authors declare no conflict of interest.

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